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Revenue accruing to the Society from subscriptions will be wholly utilized for the support and enlargement of the Journal.

CONTENTS

THE ABSORPTION OF QUININE SALTS FROM ISOLATED INTESTINAL LOOPS OF DOGS

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The rate and degree of absorption of therapeutic agents from the intestinal tract of the patient has always been a matter of speculation about which we have very little exact information. Numerous studies of absorption rates of the normal products of digestion of foodstuffs have been carried on, but much less has been done concerning even the commonest of drugs. The rate of absorption of quinine in the form of its various salts and of other cinchonas of antimalarial value is now attracting more and more attention and the present studies were undertaken in the hope of elucidating certain parts of the mechanism of quinine absorption. Such studies can take the form of measurements of the rate of disappearance of quinine from the intestinal tract, or they can be followed by the application of analytical measurements to the resulting content of quinine in the blood of the patient or animal. A further method which has been employed has been that of measuring the output of quinine in the urine. Measurements of either blood or urinary levels of quinine labor under the obvious disadvantage that they do not distinguish between the rate of absorption of the drug from the intestine and the rate of its metabolic decomposition. Since we are as yet unable to say whether the true antimalarial effect is due to the content of unchanged quinine in the blood, or to the content of some metabolite of this drug, and since the blood level is obviously the resultant of both the income due to absorption and the outgo due to both metabolic decomposition and urinary excretion, measurements of blood levels of quinine resulting from dosage by mouth provide results complicated by a great many factors. In addition we are not able to say to what extent measurement of the degree of urinary excretion of unchanged quinine can be correlated with antimalarial efficacy. These uncertainties are further complicated by the fact that our best methods for the determination of the small amounts of quinine in urine, and particularly in blood, are far from being specific methods. Whether based on the production of a turbidity due to some such precipitating agent as silico-tungstic acid, or to the ability of the quinine to fluoresce under certain conditions,

or to some other method based on a colorimetric reaction, our methods, while often of a sufficient degree of accuracy, are all under distinct suspicion of lack of sufficient specificity. We do not know to what extent quinine metabolites of similar composition may react with our commonly employed analytical reagents and we can therefore not make unequivocal statements that the results of such analytical methods when applied to blood or urine or animal tissues may not represent in reality quinine plus some metabolite in unknown proportions. For these reasons we feel that some virtue attaches the direct determination of the rate of disappearance of quinine salts under various conditions from the animal's intestinal tract.

Such determination as applied to experimental animals may take two general forms. The drug can be introduced into the gastro-intestinal tract and after a given period of time the animal can be quickly killed, opened, the gastro-intestinal tract tied off, both at esophagus and anus, and the remaining amount of drug can be determined in the washed out contents. This is the technique applied by Cori to the determination of various absorptions from the gastro-intestinal tract and is usually applied in work with rats. There are many experimental difficulties which cannot be discussed here and the method labors under the disadvantage inherent in the variations between individual animals, since only one experiment can be done with each individual. It does however provide a means of measuring the absorptive capacity of the whole intestinal tract. The other method, frequently used, is that of providing the experimental animal (usually a dog) with an isolated intestinal loop of the Thierry-Velly type, as perfected by Ravdin and Johnston. These loops have the distal end closed and the proximal end brought out to an opening in the abdominal wall. The loop retains its own blood and nerve supply. The remainder of the small intestine is anastomosed. Since the loops are commonly about one foot long, the animal still has a sufficient length of small intestine and subsists for months or years on a normal diet, while the excised loop, generally taken from the jejunal region, can be used for repeated experimentation. By means of a closing system of rubber balloons and tubes various doses of the substance under examination can be placed in the loop and, after a given length of time, washed out in semi-quantitative fashion, the difference being that absorbed. There is thus eliminated any question of direct fecal loss. While such an arrangement employs only a small proportion of the total absorbing surface of the animal, it does make possible comparisons in the same animal of the rate of absorption of a number of different compounds or of the same compound

under different conditions. Such animals have been used over periods of months or years, and while definite lowering of the absorptive ability of the loop has been detected over a period of about two years in such animals, series of absorption experiments can be carried on for periods of months without there being any detectable change when some standardized procedure is applied.

We have already published the results of some preliminary comparisons of the rate of absorption of quinine sulfate and quinine hydrochloride from such loops. During the early stages of the absorption (up to 30 minutes) the hydrochloride appears to be definitely more readily absorbed than the sulfate. This might have been expected as a result of the much greater water solubility for the former. However, investigations carried on some years ago at the University of Pennsylvania on the absorption of such an insoluble amino acid as cystine showed that this substance was absorbed from such loops at the same rate; whether applied as the very insoluble isoelectric material in water suspension, or as the very soluble sodium salt. It appeared that the amino acid left the intestinal loop at a rate specific for itself without regard to water solubility and that the absorption of the sodium salt of cystine was equivalent to offering the intestine equimolar amounts of alkali and of the amino acid, and that each of these was absorbed at its own specific and independent rate. We were therefore much interested to find indications of a difference in the rate of absorption of two salts of quinine, one highly water soluble and the other not. It might of course be argued that the mechanism of absorption of an amino acid, a natural product of protein digestion might well differ from that of a drug like quinine, which is to the animal organism essentially a "foreign body". The fact that water solubility seemed to play a comparatively small part in this absorption was further indicated by the fact that quinine sulfate, although quite insoluble in water, becomes much more soluble in increasing concentrations of sodium chloride. The maximum solubility is reached at about four percent after which further increases in the concentration of sodium chloride cause a sharp decrease in solubility. At physiological saline concentrations the solubility of quinine sulfate is approximately twice its solubility in pure water. We were therefore much interested in comparing the relative absorption of quinine sulfate placed in the loop as a water suspension with that introduced in solution in various concentrations of sodium chloride. We found, however, within the limits of experimental error, no difference between the rate of absorption from water and from the sodium chloride solution. On the assumption that such lack of difference might be ascribable to the much more

rapid removal of sodium chloride from the loop, we increased the concentration of salt up to the maximum of the solubility curve (about four percent NaCl) in the hope that as water was being taken into the loop to dilute this very hypertonic solution, and as some sodium chloride was being lost, a point would be reached at which more rapid disappearance of the drug from the loop could be demonstrated. However, the only result obtained was a *lowering* of the rate of absorption when these higher concentrations of sodium chloride were employed. There appeared to be no concentration of sodium chloride at which its ability to dissolve large amounts of quinine sulfate reacted in any favorable way. Such results would of course somewhat confirm the absorption picture which we had set up as a result of the earlier experiments with cystine.

The fact that the total amount of absorbing surface available is not the limiting factor as regards blood levels of quinine seems indicated by certain other experimental findings. For example, some earlier work from our laboratories by Dr. Bailey D. Webb on the relative rates of absorption of quinine sulfate from the intact intestinal tract of dogs, both when normal and with moderate hookworm infection, showed little or no detectable difference in the levels of blood quinine attained from standard dosage under these two conditions. Evidently the damage done to the intestinal tract by hookworm infection did not reduce the ability of the intestine to absorb the drug, and was therefore not a limiting factor. We find similarly that absorption from isolated intestinal loops gives about the same levels of blood quinine, within the limit of experimental error, as those attained in normal animals from an intact intestine. Here again we are led to the conclusion that the amount of absorbing surface available is not at all the limiting factor, if judged by the resulting blood level. It is of interest to note in passing that even from these loops the amount of quinine which cannot be recovered from them after the experiment is terminated, and which therefore must have been absorbed. can be accounted for in final urinary excretion, even over a period of forty-eight hours to the extent of not more than about 25 percent.

Further elaboration of this work on the comparative rate of absorption of quinine sulfate and quinine hydrochloride from the same dogs has led to some other very interesting results. For example, if the experiments are prolonged, the differences between the rate of absorptions of these two salts become very much less, certain animals showing less difference between these two rates than others. In any case, as the period of time for which the dose remains in the loop is lengthened to periods from 60 to 90 minutes, less sus-

tained differences can be found between the hydrochloride and the sulfate. If these curves for percentage absorption versus time drew in together at approximately 100% absorption of the drug no one would be surprised. Such a result would in fact be the only possible one. However, we have been surprised to note that curves for both of these salts reach an approximate maximum when only about 30% of the drug has been absorbed, thus leaving about 70% to be washed out of the loop. This behavior implies the existence of either some physical mechanism which limits absorption or of some physiological change in the intestinal wall. We must also consider the possibility of factors affecting conditions in the intestinal loop which might not apply in the intact animal. The hypothesis has been advanced that the pH of the contents of the intestine is such as to convert at least a portion of the quinine salt into the free base and that the latter might be more slowly absorbed. However, we have compared the rates of absorption of quinine free base to quinine sulfate in the same loop for the same period of time and have found that for 30 minutes the rate of absorption of the free base is from 2 to 3 times that of the sulfate. This would indicate a possible advantage in the use of the free base but it remains to be seen whether or not the same results are obtained from the whole G. I. tract of an intact animal. We plan to investigate this point next by the Cori technique.

A recent paper by Dr. Haag presents data showing that the acid-base balance of the animal body affects the percentage urinary excretion of standard dose of quinine. On alkalization the urinary excretion was about half the value attained after acidification with ammonium chloride.

We have been interested to investigate whether such a result could be attributed to any effect on absorption and have compared for both quinine sulfate and quinine hydrochloride the rates of disappearance from the loop under normal conditions, after dosage by mouth with ammonium chloride and after similar dosage with sodium bicarbonate. In each case the treatment with acid or alkali was continued until a marked effect on urinary pH was attained before the quinine salt was introduced into the loop. In neither case was any effect whatever observed on the percentage of the drug absorbed. For example, a 30-minute experiment with quinine dihydrochloride showed, after ammonium chloride administration, 29.8% absorbed and after sodium bicarbonate, 29.5%.

Introduction of the ammonium chloride into the loop with the quinine salt also produced no change in percentage absorption. Introduction of sodium carbonate into the loop with quinine hydro-

chloride changed the latter to the free base and produced values for percentage absorption higher than those for the sulfate but of about the same order of those for the unchanged hydrochloride.

Comparisons have also been made of the rates of absorption from the same loops of the dihydrochlorides of cinchonine, cinchonidine and dihydroquinidine. As compared with a value of 25% absorption for quinine dihydrochloride, the other alkaloids gave, under the same conditions, the following values:

	(30 *min.*)	(60 *min.*)
Cinchonidine HCl	16.3%	35.6
Cinchonine HCl	38.2%	64.3
Dihydroquinidine HCl	34.4%	
Totaquine HCl	35.6%	

There are obviously many incomplete features of these data which we hope to fill in, after which we plan to seek confirmatory evidence by the Cori technique using the entire G. I. tract of intact animals.

STUDIES ON THE PERIODICITY OF INDUCED *PLASMODIUM VIVAX**

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It has been generally considered that paroxysms of *Plasmodium vivax* exhibit a periodicity of 48 hours. However, it became apparent sometime ago that the strain of malaria used at the South Carolina State Hospital routinely for the therapy of neurosyphilitics exhibited a cycle that was shorter than 48 hours. The present work was undertaken to determine the periodicity of this strain and to compare it with other strains of *P. vivax*.

MATERIALS AND METHODS

The majority of the observations were carried out on the St. Elizabeth strain of *P. vivax*. This strain is widely used for the treatment of neurosyphilis and our method of inducing it is described elsewhere (Mayne and Young, 1941).

In studying the synchronicity, patients showing paroxysms on alternate days (so-called tertian) were chosen, as this indicated only one brood of parasites were present. Thus, the measurements were easier to make. Slides were made daily except in studying the stages of the parasites; then they were made at four-hour intervals except just preceding the expected fever rise, when they were made hourly.

Measurements of the intervals between fevers were made from one fever peak to the following peak. This interval is designated in this report as the "paroxysmal interval." As the temperatures were taken every four hours and hourly when over 100° F., the peaks of the fever were evident. When the fever remained at peak for more than one reading, the first reading was considered as the peak. As the fever peaks were shown to have a definite time relationship to the segmentation phases of the parasites, the use of the fever peaks as a measure of the asexual cycle of the parasite was convenient.

OBSERVATIONS

On several patients the relationship of the segmentation of the parasites to the fevers was measured. It was found that the peak of the segmentation preceded the peak of the fever by several hours. The peak of the fever coincided closely with the peak of the young

ring stages. This is very similar to the sequence of events in *P. malariae*. (Young, Stubbs and Coatney, 1940).

By knowing this relationship of parasite stages to fever peaks, it is clear that the time interval between fever peaks can be taken as the measure of the length of the asexual cycles.

Four hundred and one readings of the paroxysmal interval were made on the St. Elizabeth strain, involving 41 patients. These data are shown in Table 1. The range extended from 31 to 57 hours.

Table 1.—*The Means and Standard Deviations of the Periodic Fevers in Blood and Mosquito Induced Infections (St. Elizabeth Strain)*

Method	Number	Mean Length	Standard Deviation Individual Length	Standard Deviation Mean Length	$\frac{\text{diff}}{\sigma\text{diff}}$
Blood	307	43.3811	3.3172	0.1322	0.4465σ
Mosquito	94	43.5532	3.5087	0.3619	
Total	401	43.4214			

Usually the low and high extremes of this range occurred consecutively, indicating an irregular reaction which was compensated for in the following paroxysm.

As shown, the average length of the paroxysmal interval was 43.42 hours (43 hours, 25 minutes).

Of these 401 readings, 307 were made on blood induced infections and 94 on mosquito induced infections. As shown in Table 1, the average difference of the paroxysmal interval between these two groups was only 0.17 hour (10 minutes). It was found that the difference between these two groups was only 0.45 standard deviations, which indicated that it was of no significance.

A second strain of malaria was studied. This was obtained from a soldier who contracted the infection in the New Hebrides. It was transferred by blood inoculation to two neurosyphilitics here. Twenty-five paroxysmal intervals recorded averaged 45.76 hours (45 hours, 46 minutes). These were tested statistically against the blood induced infections of the St. Elizabeth strain and the difference

Table 2.—*The Means and Standard Deviations of the Periodic Fevers of the New Hebrides and St. Elizabeth Strains (Blood Induced)*

Strain	Number	Mean Length	Standard Deviation Individual Length	Standard Deviation Mean Length	$\frac{\text{diff}}{\sigma\text{diff}}$
New Hebrides	25	45.7600	1.65	0.33	6.7σ
St. Elizabeth	307	43.3811	3.3172	0.1322	

(6.07 standard deviations) is apparently significant (Table 2). Although the sample from the New Hebrides strain is small, this dif-

ference tends to indicate that different strains might show a characteristic periodicity.

Through the courtesy of Dr. J. E. Moore, the *vivax* strain used by him was studied. Readings of the paroxysmal intervals were made on ten of his patients in Baltimore and from two patients who received that strain in South Carolina. Sixty-four paroxysmal intervals averaged 41.52 hours (41 hours, 31 minutes). This, too, is a small sample, but the trend supports the other evidence of *vivax* infections showing periodicities of less than 48 hours.

In addition to the above data, published reports reproducing temperature charts of *vivax* infections were studied. One of these was published by Marchiafava and Bignami in 1894. These charts indicated that the periodicity is shorter than 48 hours.

DISCUSSION

Evidence from the St. Elizabeth strain of *vivax* malaria at our laboratory indicates that the life cycle of the asexual parasites, as measured by the paroxysmal intervals, is less than 48 hours. The strains obtained from the New Hebrides and from Baltimore, Maryland, also exhibited a periodicity of less than 48 hours.

Although it has been convenient to think of parasitic life cycles in warm blooded animals as occurring in multiples of 24 hours, there is no apparent reason that this necessarily should be true for all species of parasites. Coatney (1940) found a species of bird malaria, *P. relictum,* which exhibited a 27-hour cycle.

Some observers are inclined to believe that continued passage by blood inoculation might alter the characteristics of malaria infections. That such did not influence the present findings on the periodicity is indicated by two factors: 1. In the St. Elizabeth strain there was no significant difference between the blood induced and the mosquito induced infections; 2. the New Hebrides strain, which apparently had been through only one blood transfer, also showed a life cycle shorter than 48 hours.

The malarias tested here originated from three sources and might prove to be separate strains. It is quite likely that the one from the New Hebrides is a different strain from the St. Elizabeth strain. Although a large number of observations were not made on the New Hebrides and the Baltimore, Maryland strains, these showed periodicities of 41.52 and 45.76 hours, respectively. This suggests that each strain might have a characteristic periodicity. If so, this will be a valuable point in distinguishing strains.

SUMMARY AND CONCLUSIONS

1. Observations were carried out on induced *P. vivax* malaria to determine the periodicity of the paroxysms and the relationship

of the segmentation of the parasites to the fever. A total of 490 fever intervals were studied in three strains of malaria induced in 53 neurosyphilitic patients.

2. The peak of segmenting parasites occurs several hours before the fever. By the time the peak of the fever is reached, the segmentation process is virtually over.

3. Four hundred and one measurements of intervals between paroxysms were made on the St. Elizabeth strain and these averaged 43.42 hours (43 hours, 25 minutes). Three hundred and seven of these were from blood induced infections and 94 from mosquito induced. No significant difference between these two groups was found.

4. Measurements upon infections of *vivax* malaria from other sources were made. A New Hebrides strain of *vivax* showed a periodicity of 45.76 hours and a strain from Baltimore, Maryland, showed a periodicity of 41.52 hours.

5. None of the strains tested showed the 48-hour periodicity that *vivax* malaria is supposed to exhibit, but rather a shorter periodicity. Neither was evidence of a regular 48-hour periodicity discovered in various published fever charts, some of which dated back to 1894.

6. It is suggested that the length of the asexual cycle, as shown by the periodicity of the fevers, might be a strain characteristic.

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METHODS OF HANDLING AND FEEDING *ANOPHELES QUADRIMACULATUS* SAY UPON MALARIOUS PATIENTS*

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Because of an increased need for infected mosquitoes at the Malaria Research Laboratory, it has been necessary to find more efficient ways of handling and infecting mosquitoes.

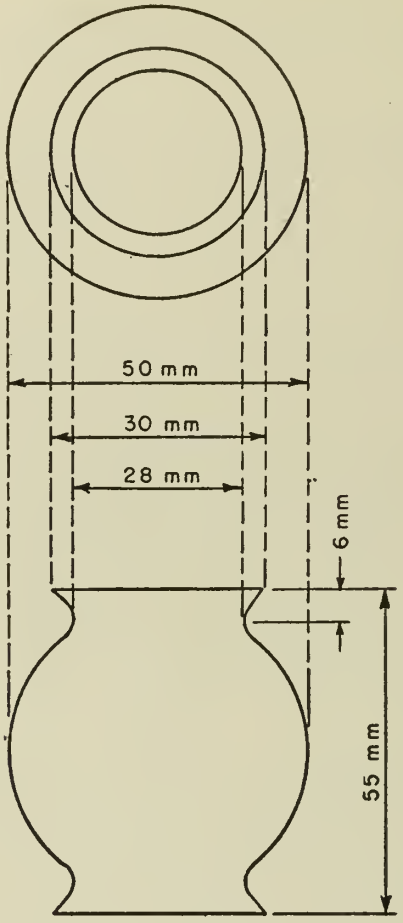
During the year ended June 30, 1943, about 100,000 specimens of *Anopheles quadrimaculatus* were reared in the insectary. Approximately 14,000 of these were applied to malarious patients. Much of the following information on the feeding of mosquitoes was gathered during this period.

FEEDING JARS

The mosquitoes to be fed upon patients were housed in small glass cages. These cages were designed about eight years ago by the late Dr. Bruce Mayne. The shape and dimensions of the jar are shown in Figure 1. Bobbinet of a mesh of 16 to the inch is cut in squares slightly larger than the end of the jar and attached to the neck of the jar by rubber bands. In the course of feeding, these bobbinet covers become soiled but can be washed out and used repeatedly.

A mosquito is placed in the jar soon after emergence and remains therein until removed for dissection. Pertinent data are written on a small label attached to the outside of the jar. As many as five mosquitoes can be placed in each jar and kept satisfactorily. However, it is our impression that best results are obtained when there is only one mosquito to a jar.

There are several advantages of this small jar over other containers which have been used here. It is quite easy to observe the mosquito from any angle. It is easier to see through the sides of the jar than it is through the netting. Furthermore, the curvature of the sides tends to magnify the mosquito, which is of aid particularly when one wishes to observe carefully the feeding process. The jars are light (not over one ounce), easy to keep clean, and require little



NOTE – THE LIPS AT BOTH ENDS ARE ADAPTED TO ACCOMMODATE A PIECE OF CLOTH NETTING HELD IN PLACE WITH RUBBER BANDS

Figure 1. Glass cage for use in experimental feeding of a single insect.

space. By the use of a four-drawer storage cabinet, 400 of these jars can be stored in a space of 26x20x24 inches.

AGE AND PREFEEDING TREATMENT OF THE MOSQUITO

The effect of age and prefeeding are so closely inter-related that they must be considered together.

The pupae were collected about the same time each morning and this was used as the starting point for the measurements. Therefore a discrepancy of 24 hours might occur in the ages. The prefed

mosquitoes were offered sugar solutions (called "prefeedings") daily from time of emergence to the blood meal. The not-prefed specimens received only water.

The effects of age and prefeeding lie in the influence on mortality prior to the blood meal, the number of surviving mosquitoes which actually took a blood meal, and the percentage of emerged mosquitoes which survived and took a blood meal (effective percent fed).

The data bearing on these points are shown graphically in Figure 2. The mortality prior to the blood meal was higher in the

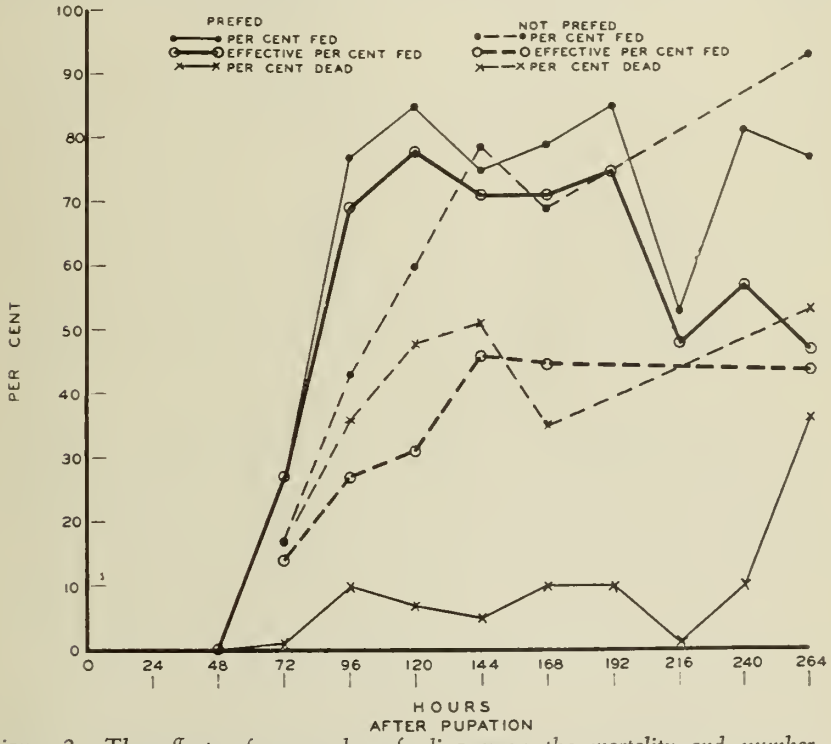


Figure 2. The effects of age and prefeeding upon the mortality and number of mosquitoes taking blood meals, as well as the percentage of emerged mosquitoes which survived and took a blood meal (effective per cent fed).

not-prefed mosquitoes than in those which were prefed on sugar solutions.

The actual number of mosquitoes feeding as well as the "effective percent" feeding was higher in the prefed group than in those not-prefed.

An experiment was then set up to determine the optimum age after emergence and the amount of starving necessary to obtain best blood feeding. It was found that the optimum blood feeding was

obtained between 49 and 72 hours after emergence with the mosquitoes having been fed on sugar solution not over 12 hours previously. (Figure 3).

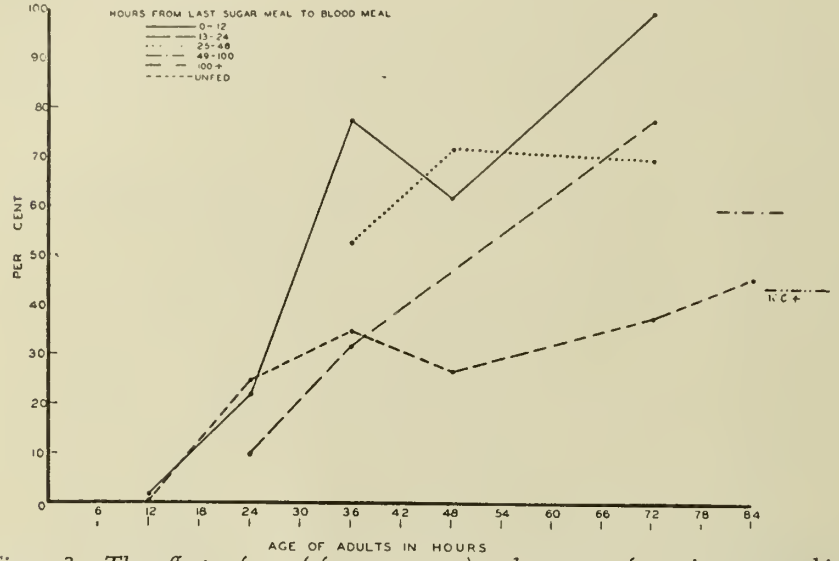


Figure 3. The effects of age (after emergence) and amount of starving upon taking a blood meal.

EFFECT OF MATING

Several times note was made that certain batches of mosquitoes fed very poorly. Search of the records indicated that these had been placed in individual bottles very soon after emergence. This indicated that perhaps no time for mating had been allowed and that this might be the influencing factor. Accordingly, about half of one batch of pupae were placed individually under small jars in a pan of water. Thus each mosquito emerged without possible contact with another. The rest of the pupae were allowed to emerge into a cage of sufficient size to allow mating. Both sets were treated and fed in exactly the same manner. This experiment was run twice. These results indicated that those "exposed" (to mating) fed better than the "virgins" by a ratio of 84% to 58%. Furthermore, the mortality seemed slightly higher in the "virgins."

CONDITIONS PREVAILING AT TIME OF FEEDING

The conditions of temperature, light, humidity, and of the patient at the time of feeding are probably not as important as is often supposed. Experience at the Columbia laboratory indicates that unless extreme environmental conditions prevail, the condition of the mosquito and its appetite are the primary factors. Of course a patient who is extremely nervous, frightened, or suffering a chill

may actually prevent satisfactory feeding because of his movements. Also there are indications that at times a slightly deleterious effect is exerted by a high intensity of illumination.

The effects of room temperature within the ordinary range in relation to feeding seem to be slight. Table 1 contains ten feeding

Table 1.—*Effect of Temperature Upon Feeding. (Ten Batches Fed in Each Temperature Range).*

Ward Temp. °F.	Number Tried	Range of Feeding Per Batch in per cent	Number Fed Per cent
68 - 74	1440	56 - 92	77
75 - 79	1191	73 - 93	79
80 - 84	1091	63 - 98	89
85 - 90	1004	67 - 97	84

trials for each of four temperature ranges and does not indicate any great difference.

To determine roughly the effect of light intensity, some simple trials were run involving about 1000 mosquitoes. The side of the patient next to the window was called the "light" side and the side away from the window the "dark" side. The mosquitoes on the dark side fed better than those on the light side by the ratio of 69 to 55 percent. Covering the mosquitoes with a sheet increased the feedings on the light side by 9 percent and on the dark side by only 1 percent. In another series, involving about 1000 mosquitoes, the covered mosquitoes fed better than the uncovered by the ratio of 79 to 71 percent.

The above indicates a slight advantage to be gained by feeding in light of lower intensities. Covering the mosquitoes is usually used only when feeding is abnormally slow.

ROUTINE PROCEDURE

The technique for the preparation and feeding of *A. quadrimaculatus* on malarious patients has been made to incorporate the above factors. Briefly, the following procedure is used:

The pupae are collected daily at about 9:00 A. M. and placed in a half-pint cardboard food container under a bobbinet-covered lantern globe. Emergence usually starts about 30 hours later and continues for about 30 hours. Cellu-cotton pads soaked in syrup solution (5% dextrose or "Karo") are placed on the jars each evening and removed each morning. (This feeding procedure is followed for all adults both before and after their infectious bloodmeal).

When emergence has been completed the adults are transferred into a 10"x10"x15" glass cage. This cage has circular end-openings

fitted with cloth sleeves. The mosquitoes are left in this cage for a period of from 24 to 48 hours to allow mating (mating has been observed in this cage).

Removal of the female mosquitoes into either the stock cage or into the small feeding jars is done with a so-called "aspirator" or sucking tube.

The feeding jars are transported to the scene of the feeding in a metal box lined and covered loosely with towels. This protects them against breakage, desiccation, and the elements enroute.

Preparation of the patient consists in placing him on his back on a bed with the top sheet crumpled between his legs and over his loins and smoothed out again over his torso. This crumpled sheet serves as a support to hold the jars snug to the insides of the legs. The jars are placed snugly against both sides of the patient's legs. Those on the outerside of the legs are held in place by the natural sag of the bed.

Occasional tapping on the outer end of the jar may be necessary to activate some of the more sluggish mosquitoes. Feeding may be slow and a sheet covering required. Or it may be very rapid and practically all mosquitoes attack the patient immediately. A recent feeding without cover resulted in 97% fed to repletion in 5 minutes. As many as 400 mosquitoes have been fed in 45 minutes by the above method. Ordinarily not over 45 minutes is allowed for feeding a batch of mosquitoes.

At the time of feeding a standard thick-thin blood smear is made for purposes of record and prognostication.

When feeding is completed the mosquitoes are replaced in the box and returned to the insectary, which is temperature and humidity controlled. Here they remain, receiving daily feedings of syrup until dissection indicates infection.

SUMMARY

Methods of handling *Anopheles quadrimaculatus* to secure the feeding of large numbers on malarious patients are described.

1. A small glass jar with the ends covered with bobbinet is used. From one to five (preferably one) mosquitoes are placed in each jar.
2. In the preparation of the mosquito, two factors appeared definitely to enhance feeding:
   a). Age of mosquito. At least 48 hours should elapse between emergence and application to the patient. The optimum time seemed to be between 49 and 72 hours after emergence.

b). Prefeeding. Mosquitoes prefed on sugar solutions took blood meals more readily and lived better than those not prefed. Sugar solution should be offered the insect daily and should not be removed finally more than 12 hours before the infective meal.

3. Mosquitoes which had had the opportunity to mate fed better than those which had not.

4. Room temperature changes within the range of activity of the insect seemed to exert little effect. Some increase in feeding was found by reducing the intensity of light.

THE ENTOMOLOGICAL PHASES OF MALARIA CONTROL PROGRAMS

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Literature abounds with discussions of the possibility and practicability of the control or eradication of malaria by medication of the human host. However, the therapeutic, immunological or other methods to accomplish this end have yet to be found and therefore the time honored attack, that of the control of the insect vector, must still be considered the most effective means for reducing the human malaria infection rate.

Insect control measures, to be effective and economical, must be based on the biology and habits of the particular species involved. The determination of the dangerous species and the study of its habits are entomological functions whether performed by the entomologist or by those of other disciplines. This is not a discussion of the place of the entomologist on malaria control programs but rather of the use of entomological findings and methods on a task which essentially lies within the field of economic entomology.

The first important contributions to mosquito biology in this country were made by Dr. L. O. Howard (1, 2), who in 1900 published a bulletin entitled "Notes on the Mosquitoes of the United States" and later in 1901 a more comprehensive volume entitled "Mosquitoes: How they live: How they carry disease: How they are classified: How they may be destroyed". These studies, which were prompted by the then recent incrimination of mosquitoes in the transmission of yellow fever and malaria, gave only an inkling of the variety of mosquito species, the diversity of their habits and the complexities of their control. They did, however, make information on the differences between anopheline and culicine mosquitoes generally available. In spite of this, mosquito control programs undertaken for malaria control in the United States in those early days seem to have been prosecuted indiscriminately against all types of mosquitoes.

The first control work directed solely against Anopheline mosquitoes for malaria control appears to have been that done by Herms (3) at Penryn, California in 1910. This marked the beginning of the use of anti-anopheline rather than general anti-mosquito measures in malaria control in the United States. In 1912, Carter and

Von Ezdorf of the U. S. Public Health Service, initiated studies and practical demonstrations of malaria control in the south in which anti-anopheline measures were advocated and this constructive work laid the groundwork for the extensive projects which have since been prosecuted.

It was about this same time (1910) that Darling (4), working in Panama, found that all species of anophelines are not alike in their ability to transmit malaria and that *Anopheles albimanus* was the chief malaria transmitter of that region. From this important discovery was evolved the principle of "Anopheles-species control of malaria" or "species-sanitation" as it is sometimes called. By the application of this principle, as was done in the Canal Zone with marked success, malaria mosquito control can be limited to the vector or vectors and results obtained with a minimum of effort and expenditure, thus putting the work on an economical basis. However, in order to do this selective work it is imperative that careful entomological studies precede inauguration of the work and that supervision be maintained.

In spite of the successful experience with species sanitation in Panama, this method of malaria control has been slow of adoption in the United States. This seems to have been attributable not so much to a lack of information on the species of anophelines involved in malaria transmission and their habits as to a desire on the part of those charged with the work to cater to popular demand for general mosquito control. Measures directed against all anophelines or against all mosquitoes, if successful, will produce results in malaria control and this is logically the reason for a lack of interest in refinements of procedure. General mosquito eradication programs are of great public benefit and are desirable where money is available for this expensive work. However, it is quite probable that malaria control, which involves much simpler and less expensive operations, has been greatly retarded because of these attempts at all-inclusive mosquito-control. It was not until 1937, when the boards of health of a number of southern states employed entomologists, that much attention was given species sanitation on a wide scale in malaria control work in this country. Provision for the employment of these men and also for their training under specialists of the U. S. Bureau of Entomology and Plant Quarantine, the Rockefeller Foundation and the Public Health Service was made possible by expenditures of funds allotted to the States by the U. S. Public Health Service. A survey of the work of these men made by the writer in 1939 (5) indicated that about 50% of their time was being spent in field malaria mosquito surveys, 17% in research on

mosquitoes and the remaining 33% included supervision of field control operations and educational work on malaria and mosquito control. The employment of these entomologists marked the beginning of a period of progressively increasing application of the accumulated knowledge of anopheline bionomics to malaria control work.

With the advent of the present war, cognizance was taken at once of the need for entomological work in malaria control by both military and civilian health authorities. The number of entomologists who had even a smattering of knowledge of mosquito biology and control was, of course, inadequate to meet the demand for these services. However, for those who have had broad entomological training, the transition from work on one group of insects to another is not difficult and it is certain that those who have lately taken up work in this field have contributed greatly to the efficiency of malaria control.

The entomological phases of a malaria control program are those which have to do with the insect vector. Presupposing that a locality is known to be malarious or is epidemiologically suspected, it is the function of the entomologist first to make a thorough entomological survey of the area. This includes determination of the local anopheline fauna and the relative abundance of the various species which is done by collecting adults from a good series of diurnal shelters, by the use of bait and light traps and by records of biting. Records should be made of the location at which these data are collected, of the types of shelters and of traps used, and the length of time over which biting observations are made. The numbers of each species of *Anopheles* taken in each case should be carefully recorded so that with subsequent accumulation of data from these same places reliable evaluations of the effect of control work can be made. Density observations both within the control area and in similar adjacent areas which will not be affected by control work are necessary so that measurements of progress can be made on the basis of normal seasonal abundance of the vectors.

In case the vector is not known, the next step is to determine which of the local species are harboring malaria plasmodia. This technical work, which involves careful handling and dissection of mosquitoes is logically a job for the parasitologist, and if one is available the work of the entomologist in this connection may consist only of obtaining the necessary specimens and field data. With the results of this study at hand, that is, the naming of the vector species, it is possible to proceed with the formulation of plans for the control of these individual species, or in other words, to practice species-sanitation. This requires, first of all, the finding of their breeding

places. To do this, the best available map should be secured and a reconnaissance of the area made to locate as far as possible all waters within a radius of the population to be protected, the length of which will depend on the flight range of the particular vector concerned. Anophelines being as a rule weak fliers, this distance can be approximated at one mile for preliminary work. The water areas should be spotted on the map and each searched for the occurrence of larvae. Mapping and searching for larvae may of course be done concurrently. Specific identification of the specimens found must be made and can be done either by rearing specimens and determination of adults or by larval identification. In practice both methods should be used to obviate chances for error. Having proceeded this far, the entomologist is in a position to name the vector or vectors involved, their abundance and their breeding places at the time of the survey. With this information at hand the problem of control can be approached from the engineering aspect in a logical manner.

As control work progresses, it is necessary that careful routine observations be made to determine the effectiveness of the work in reducing local vector densities. If these are not lowered to a satisfactory level, the reason must be found. It may lie in ineffective larvicidal work, undiscovered breeding places, formerly non-breeding areas coming into production, or unusual flights from extensive breeding areas outside the control zone. In any case the finding of the trouble will indicate the necessary remedial measures.

Anopheline mosquitoes, in common with other insects, vary greatly in their biting, associational, breeding and flight habits, not only between species, but within the individual species in different latitudes and at different seasons. Biting, associational and longevity habits, together with the customs of the human population within its range, are all-important in determining the probability that an individual species will transmit malaria; for so far as is known at least, all species of anophelines are susceptible of infection by malaria plasmodia, although some are reported to be more or less refractory to certain parasite species. Breeding and flight habits are all-important in determining the control measures for a given species. The determination of all these factors in any area and their application to control practices is the only logical approach to intelligent anopheline mosquito control. If the vector is eradicated or reduced to inconsequential numbers, we know that malaria will be controlled. Reports on miles of ditches dug, gallons or pounds of larvicide used or money spent give an index only to the size of the job done. The contribution of this work to malaria control must be

measured by the actual reduction in the vector species accomplished.

At these meetings last year a paper was presented which detailed the entomological procedures used for guiding anopheline control work on the Malaria Control in War Areas program being prosecuted by the U. S. Public Health Service in cooperation with State Boards of Health (6). The following of those procedures has resulted in a close integration of the work of the engineer and entomologist in planning and instituting control measures and in evaluating their results. This has placed control work on a sound basis. Whether on a large project or a small one, the criterion of satisfactory work is the reduction and maintenance at low levels of malaria vector populations. Success or failure of a malaria mosquito control project is determined by the answer a supervisor can give to our universal query, "what are your density counts," or more familiarly in the South at least, "what are your 'quad' counts?"

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EDUCATIONAL FACTORS IN THE ULTIMATE CONTROL OF MALARIA

Presented at National Malaria Society, Cincinnati, November 17, 1943.

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We look upon malaria as the result of the complex inter-relationships between three living organisms: *Plasmodium vivax, Anopheles quadrimaculatus* (or other species), and *Homo sapiens.* We speak of man here as a biological organism that is not simply the victim of a parasitic disease, but is indeed an essential element in the problem, just as truly as is the parasite or the mosquito. Any effort to break the malaria chain must be based upon an understanding of these inter-relationships—whether the attack is on the parasite by drugs (through man), against the larval or adult mosquito, or against the third member of this unholy "triumzoate" (we can't say "triumvirate"), man himself.

Unlike the mosquito, man can't be conveniently eliminated. The attack against man's part of the problem is the attack against ignorance. Modification of man's behavior may be as effective as elimination of the mosquito. Man is the only one of the three organisms that can exercise volition in breaking the chain. *Homo sapiens* is part of the problem not only as individuals harboring the parasite, but as individuals and groups cooperating with the mosquito in transmitting the parasite.

The attack on ignorance requires techniques for transmitting information. Three recognized fields of activity in health education are: Professional and Technical Training, Regular Educational Channels and Information to the General Public. These contribute both to organized malaria-prevention activities by Public Health and other agencies and to individual anti-malaria activities based on sound information.

Homo sapiens has developed, as one of many social achievements, health agencies which have borne the responsibility for preventing diseases, including malaria. The arrow in Figure 1 represents the efforts of health agencies—local, state, federal and private—to prevent malaria. This effort is only partially successful due to the blocking effect of man-made and man-tolerated malaria. The hazard of malaria in the United States is due in large measure to this influ-

ence, which in turn is due to inadequate preventive practices in various fields of human endeavor. Health agencies cannot possibly

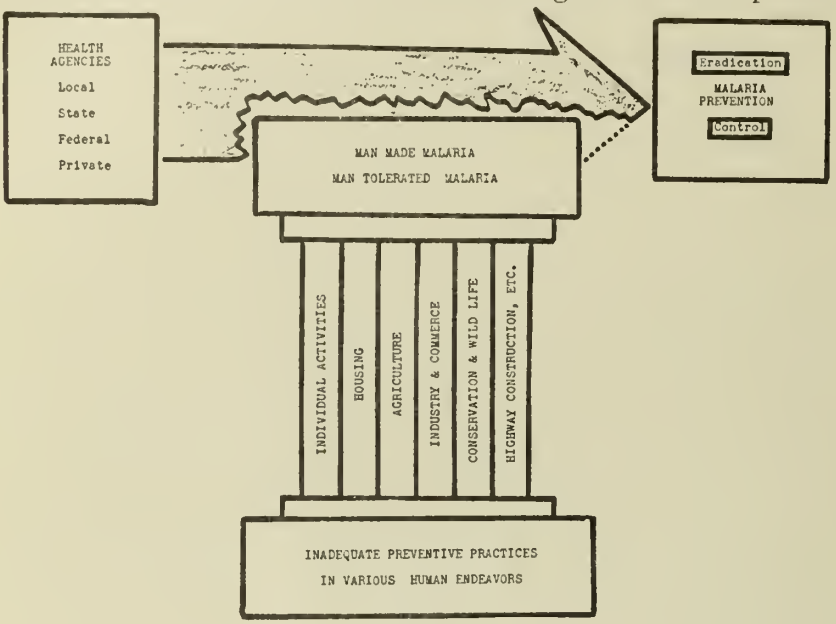

Figure 1

overcome the influence of this factor alone. The causes of man-made malaria have their origins in many activities, all of which must contribute if malaria is to be prevented.

We use the word "prevention" rather than "control." "Control" carries a slight connotation of willingness to accept the continued existence of the disease as inevitable. We are aiming at "malaria *prevention*" at present principally by methods of "mosquito *control*." "Eradication," on the other hand, if achieved, is suggestive of an optimism which may allow decreased vigilance in preventive effort. Not so. Malaria prevention must be practiced even in the complete absence of the disease in order to "keep it eradicated." "Prevention" is more significant in terms of the viewpoint it represents than in the quantitative achievement of its methods.

Among the activities of man influencing the malaria problem, *Individual Activities* are of major importance. The habits of screening homes and spray-killing adult mosquitoes by the general public are considered by many experts to be factors of considerable importance in the present low malaria rates.

The science of *Housing*, both urban and rural, is an important factor.

The relation of malaria to *Agriculture* involves many phases of

a biologically complex science, as well as the more obvious relationship of drainage to mosquito-breeding.

The relationship of impoundments for *Industry and Commerce* to malaria should be as well known to industry's management as it is to the malariologist.

Man-made Anopheles breeding places, whether due to faulty engineering in *Highway Construction,* as leaving borrow pits or improperly placed culverts, or due to artificial impoundments for *Wild Life Conservation,* or to whatever cause, should be recognized as a hazard to man by the men who manage these affairs. Without impeding human progress by these other activities, the malariologists have the opportunity of suggesting sound methods of management, which will aid the prevention of malaria by avoiding the creation of Anopheline breeding places.

Upon the malaria divisions of the various health agencies rests the responsibility for taking a more aggressive lead in removing this barrier of man-made and man-tolerated malaria. The principal tools for this task are "educational factors."

Most experienced public health workers agree that legal coercion cannot be depended upon except to prevent the most flagrant abuses. Law might be likened to the ratchet-wheel in a watch—it maintains the gains made; but the mainspring provides power for movement. Proper handling of information is the mainspring in this case. The achievement of adequate preventive practices by these other agencies depends principally upon an appreciation for the significance of the total situation by individuals engaged in these other activities. The educational techniques which will succeed in placing such information and appreciations in the proper places have yet to be developed. This is a function not of an isolated group of specialists, but of every malariologist—physician, biologist or engineer—whose efforts are dedicated to the prevention of the disease.

Instead of supporting the obstructing factor, "man-made malaria," these various activities must contribute (as diagrammed in Figure 2) to the preventive effort. Health agencies alone are inadequate.

Economy as well as effectiveness must be considered. Long range economy in malaria prevention requires that sources of funds for malaria prevention must be identified with incomes or appropriations of funds for other constructive or profitable endeavors. This will be increasingly true as malaria rates fall even lower, inevitably decreasing money available from public funds for work against a disease which will be a potential rather than a present danger. Funds

for malaria prevention are a legitimate overhead expense of the various enterprises related to the problem. Translating this abstract

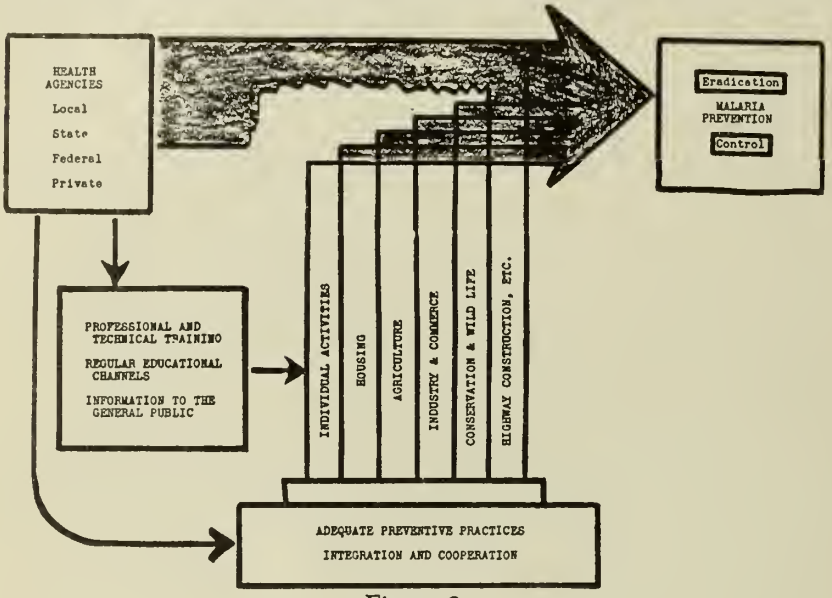

Figure 2

statement into practical action is the greatest educational task of the malariologist. To achieve it, health agencies must develop closely integrated programs in cooperation with other agencies. They must stimulate and utilize to the full the influence of the three major groups of educational activity.

The width of the arrow, in Figure 2, representing the effectiveness of malaria preventive efforts, can be greater than would ever be possible by efforts of health agencies alone.

In the United States malaria will be a problem for years to come—we hope only a potential problem, but inevitably a real one that will require consistent preventive activities by many varied groups. With the evolution of the malaria problem comes the need for shifting emphases in meeting that problem most effectively and most economically. The decades ahead will bring not only more effective methods of attacking the mosquito, and probably new drugs to attack the parasite by treatment and prophylaxis, but more than either of these will come increased effectiveness in attacking man's part of the problem—chiefly ignorance: attacking it to the extent of making complete malaria prevention a continuing reality, based upon a system of cooperative and integrated effort by many groups and individuals.

SUMMARY

1. The ultimate control of malaria implies complete and continuing prevention of the disease. In the United States we can now anticipate the actual achievement of complete prevention.

2. Viewed biologically, man is not simply the victim, but an important causative factor, both as an individual carrier, and in groups whose activities aid transmission by creating or tolerating Anopheline breeding places. In the ultimate prevention of malaria, man, or rather man's ignorance, must be a major point of attack.

3. Because of the intimate relation of etiological factors to many varied activities, prevention of malaria hazards requires conscious preventive effort by large numbers of men, individually and in groups. Malariologists and health agencies alone cannot complete the task a) because of the influence of these many activities over which they have no direct control, and b) because of economic considerations.

4. For long range economy, sources of anti-malaria funds must coincide with sources of income for other constructive or profitable enterprises, being considered a legitimate overhead expense of these activities instead of a direct drain on tax budgets.

5. Such achievement depends upon methods for informing proper persons of their opportunities and responsibilities in malaria prevention as an integral part of their regular activities. All methods of transmitting such information are included under the term "educational factors."

6. These methods are now in their early development. Techniques and materials for this task are at present woefully inadequate. This is a task for the immediate future.

7. Malariologists and health officials are the group primarily responsible for directing the development of these methods, utilizing such specialized personnel as may be required. Use of "educational factors" in preventing malaria, being a major method of attack, is the business of malariologists and cannot be left to specialized groups and thought of as entirely secondary.

8. The National Malaria Society might well afford to throw the full weight of its influence and action into immediate rapid development of this method of attack.

PRELIMINARY STUDIES ON THE FEEDING HABITS OF PACIFIC COAST ANOPHELINES

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Malaria has long been a problem for consideration in the Pacific Coast area of the United States (1, 2). Although the anopheline mosquitoes of this region have been studied in great detail systematically and biologically (1, 3-13), remarkably little has been recorded concerning their feeding habits.

In the Pacific Coast area the *Anopheles* complex is composed of *Anopheles maculipennis freeborni* Aitken, *Anopheles maculipennis occidentalis* (D. & K.), *Anopheles punctipennis* (Say), and *Anopheles pseudopunctipennis* Theobald*. Recent reviews of the relationships of the various members of this complex to the malaria problem were by Aitken (4), and Herms (14). Their conclusions were that in this area *freeborni,* which commonly feeds on man and invades human habitations, is the most important vector of malaria; *occidentalis,* as far as is known, is of little or no importance; *punctipennis,* which is largely a field mosquito and rarely invades houses, may be a vector under certain conditions; *pseudopunctipennis* on the Pacific Coast is a field mosquito and rarely feeds on man, except in the laboratory.

Results of field studies in California on malaria infection rates in mosquitoes have never been published, but infected *Anopheles maculipennis freeborni* (oocysts on the stomach wall) were collected by Herms (15) in the vicinity of Vina, California. In New Mexico *Anopheles maculipennis freeborni** were found infected by Barber, Komp, and King (16), and by Barber and Forbrich (17).

*Aitken (5) reports that within this species in California there are two varieties *Anopheles pseudopunctipennis francisanus franciscanus* and *Anopheles pseudopunctipennis franciscanus boydi.* For practical purposes of field identification females of these two types are not separable.

*At the time this work was published the North American subspecies of *Anopheles maculipennis* were not recognized, however, subsequent studies have recorded *freeborni* as the form present in this area (23).

All previous records of feeding habits have been based on studies made of house-invading habits and field observations on the tendency of species to feed on man. This study by precipitin tests on blood from engorged females collected in the field does not include a series of *Anopheles maculipennis occidentalis*. The number of samples tested from many areas is small, and will serve mainly as an indication of the feeding range of the species concerned.

The smears to be tested were mostly made from engorged females collected in domestic habitats (barnyard and rural environment). Thus man was not the dominant animal in the collecting areas but was rather one of several possible host animals on which the mosquitoes could feed.

The same methods of precipitin testing were employed as those reported in earlier papers (19, 20). Seven antisera were employed against specific serum proteins of man, horse, cow, sheep, dog, pig, and bird.*

The results of tests on the 703 smears of the present study are given in Tables 1, 2, and 3. The findings indicate that these *Anopheles* prefer domestic mammals as host animals, and that *Anopheles maculipennis freeborni* not infrequently feeds on man. These data do not permit comparison of the androphilic tendencies of the Pacific Coast anophelines because of the small numbers involved in two of the species studied. These data are only presented as showing a trend in habits, and an indication that the three species studied all normally include man as one of their host animals.

A short series of smears tested in 1942, Bang and Reeves (12) (Yakima, Washington, 1941) has been included in this study as this was the first series run on Pacific Coast anophelines. Also included is a series of smears reported in a previous paper on the mosquito vectors of encephalitis, Reeves and Hammon (19) (Yakima, Washington, 1942).

It is interesting to note that the area in which the highest proportion (7.1 per cent) of *Anopheles maculipennis freeborni* were found to be feeding on man was Marysville, California, one of the classical endemic foci of malaria in this state (Table 1).

The finding that 3.0 per cent of the 473 smears from *Anopheles maculipennis freeborni* contained human blood, would seem to support the fact that this species is involved in the transmission of malaria in the Pacific Coast area.

*This antiserum made against chicken serum was found to give positive reactions when tested against bloods from 11 other species of birds (duck, turkey, pigeon, quail, pheasant, magpie, yellow throated sparrow, yellow crowned sparrow, Brewer blackbird, rice bird, and canary). Thus it is considered that any positive reaction to this antiserum indicates an avian blood source and is not species specific.

The small series (178 smears) of *Anopheles pseudopunctipennis* tested (Table 2) are the first reported from the United States. Davis and Shannon (21) tested this species in Argentina and found 50 per cent had fed on man. Vargas (22) in studies made in Mexico found 67.6 per cent of 244 engorged *A. pseudopunctipennis* collected in human habitations had obtained their blood meals from human beings. In the present series from the Pacific Coast 49.4 per cent of the smears gave no reaction to the anti-sera employed, although the females selected for testing appeared to be freshly engorged. This group of non-reactors probably does not only represent inactivated blood meals, but also blood from animals not included in the set of anti-sera employed. Only 0.6 per cent had fed on man and the cow appeared to be its preferred host (43.4 per cent positive).

A short series of 23 smears is included from specimens collected in Arizona. *Anopheles pseudopunctipennis* has been the only common *Anopheles* found in this area, *Anopheles maculipennis freeborni* is very rare, and *Anopheles punctipennis* has not been reported (22). Malaria cases have been reported from this state and it may be that *A. pseudopunctipennis* was responsible for transmission of some of these infections.

The tests on Anopheles punctipennis (Table 3) are too few (52 smears) to be considered of great significance, but the gamut of hosts fed on and the fact that 3.8 per cent had fed on man is of interest.

SUMMARY

Precipitin tests have been run on blood from the stomachs of freshly engorged *Anopheles maculipennis freeborni*, *Anopheles pseudopunctipennis*, and *Anopheles punctipennis* collected in the field from various parts of the Pacific Coast area of the United States.

Tests were run on 473 smears from *Anopheles maculipennis freeborni* and 3.0 per cent of this group had fed on man. The highest rate for any single area was 7.1 per cent positive to human blood.

From *Anopheles pseudopunctipennis* 178 smears were tested with 0.6 per cent having fed on man.

For *Anopheles punctipennis* 52 smears were tested of which 3.8 per cent had fed on man.

TABLE I. *Precipitin Tests on Blood Meals from Anopheles maculipennis freeborni*

Area and date of collection	Number reacting to Specific Serum Protein Anti-sera								
	Horse	Cow	Sheep	Dog	Man	Pig	Bird	No reaction	Total
Yakima, Wash., 1941 (18)	45	28	0	0	0	0	0	0	73
Yakima, Wash., 1942 (19)	53	70	3	3	2(1.2%)	0	9	21	161
Marysville, Calif., 1942	35	74	3	15	11 (7.1%)	0	0	18	156
Merced, Calif., 1942	2	13	3	0	0	0	0	0	18
Corvallis, Oregon, 1942	0	0	1	0	0	0	0	0	1
Bakersfield, Calif., 1943.	0	8	30	9	1 (1.6%)	0	0	16	64
Total	135	193	40	27	14 (3.0%)	0	9	55	473

TABLE II. Precipitin Tests on Blood Meals from *Anopheles pseudopunctipennis*

Area and date of collection	Number reacting to Specific Serum Protein Anti-sera								
	Horse	Cow	Sheep	Dog	Man	Pig	Bird	No Reaction	Total
Casa Grande, Arizona, 1942	0	15	0	0	0	0	0	8	23
California, 1942	4	1	1	0	0	0	0	3	9
Porterville, Calif., 1942	0	12	1	0	0	0	1	0	14
Bakersfield, Calif., 1943	4	48	0	0	1(0.8%)	0	0	77	130
Angels Camp, Calif., 1942	0	2	0	0	0	0	0	0	2
Total	8	78	2	0	1(0.6%)	0	1	88	178

TABLE III. Precipitin Tests on Blood Meals from *Anopheles punctipennis*

Area and date of collection	Number reacting to Specific Serum Protein Anti-sera								
	Horse	Cow	Sheep	Dog	Man	Pig	Bird	No Reaction	Total
Corvallis, Oregon 1942	0	8	5	0	0	0	0	1	14
California, 1942	0	2	0	0	0	0	0	0	2
Bakersfield, Calif., 1943	0	1	0	0	1(50%)	0	0	0	2
Angels Camp, Calif., 1943	3	9	1	0	1(6.7%)	0	1	0	15
Yakima, Wash., 1942 (19)	7	10	0	0	0	0	0	2	19
Total	10	30	6	0	2(3.8%)	0	1	3	52

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A SUMMARY OF ENTOMOLOGICAL WORK AT THE FOURTH SERVICE COMMAND MEDICAL LABORATORY DURING 1943

by
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The following report is a brief summary of the entomological work pertaining to mosquito identification and malaria control in the Entomology Section of the Fourth Service Command Medical Laboratory during 1943. King and Kuhns presented a paper before the 1942 meeting of the National Malaria Society in which they described the development of the entomological services of this laboratory and also gave brief descriptions and a summary of the work done during 1942.

The entomological work during 1943 at the Fourth Service Command Medical Laboratory has been conducted along similar lines to that of 1942 except that certain improvements, based upon the experiences of the previous year have resulted in a large increase in the volume of work over that of last year, both in routine laboratory identification of specimens and the training of personnel.

The work of the Entomology Section may be divided into the following categories: (1) The mosquito collecting program, (2) The identification of specimens, (3) The preparation of mosquito collection records, and (4) The training of personnel.

MOSQUITO COLLECTING PROGRAM

A revision of the letter of instruction for making mosquito collections was prepared and sent out during February 1943 to all army installations within the Fourth Service Command which is now made up of the following states: Tennessee, North Carolina, South Carolina, Georgia, Alabama, Mississippi, and Florida. These instructions called for four classes of mosquito collections as follows: resting station, light trap, biting, and larval collections.

A map of uniform scale and with uniform symbols for indicating the locations of the different kinds of mosquito collections has been adopted throughout the Service Command. These maps were prepared during the early part of the year in each army instal-

lation and were revised when necessary by the posts concerned when changes had to be made in the locations of collecting stations. Copies of these maps were furnished the Division of Preventive Medicine of the Fourth Service Command and the Fourth Service Command Medical Laboratory by each installation so that the locations of all mosquito catches can be evaluated in respect to their locations in relation to living quarters.

IDENTIFICATION OF SPECIMENS

Only a limited number of camps have trained personnel and equipment necessary for doing their own identification of mosquito specimens; therefore this service is furnished the army installations of the Fourth Service Command by this laboratory. Mosquito specimens accompanied by collection data are mailed weekly to the laboratory either for initial identification or confirmation of identifications made by personnel at the different army posts.

A summary of the mosquitoes identified in this laboratory from January through September 1943 is shown in Table 1. The collection records clearly indicate that there was a rapid build-up in the population of *Anopheles quadrimaculatus* during July and that adults of this species gradually decreased in number during August and September. Table 2 shows the total number of adult specimens of *Anopheles quadrimaculatus* taken in diurnal resting stations, in light traps, and in night biting collections. It may be noted that the seasonal average of males and females of this species per collection was 1.50 in natural resting places, .34 in light trap collections and .16 in night biting collections. It is interesting to note that the average number of *Anopheles quadrimaculatus* per collection in resting stations during the first nine months of 1943 was 4.4 times greater than for light traps.

Mosquito collection records are not available for the first four months of 1942 so that a comparison of resting station and light trap catches for the period January through September of both years could not be made. The average number of *Anopheles quadrimaculatus* per collection was 6.4 times greater in resting stations than in light trap collections from May through September 1942, while the average for resting station catches during this same period in 1943 was 4.3 times greater than the average light trap collection.

PREPARATION OF MOSQUITO COLLECTION RECORDS

It was noted in Table 1 that weekly mosquito collections were received and specimens were identified from 142 army installations within the Fourth Service Command from January through Sep-

TABLE I. *Mosquitoes Identified at the Fourth Service Command Laboratory from January through September* 1943.

MONTHS	JAN.	FEB.	MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	TOTAL
Number of Posts	21	19	22	55	87	112	114	120	120	142
Number of Species	32	27	34	41	44	50	51	50	47	54
Number of Larvae	5,651	6,585	7,092	12,706	15,334	12,701	18,915	25,585	19,965	124,534
Number of Adults	2,849	1,963	1,287	5,322	20,820	41,905	146,400	130,964	119,812	471,322
A. quadrimaculatus (percent of adults identified)	1.4%	1.13%	0.5%	0.7%	1.1%	2.9%	10.1%	7.0%	3.3%	6.3%

TABLE II. A Comparison of Collections of Adult *Anopheles quadrimaculatus* in the Fourth Service Command from January through September 1943.

MONTHS	Diurnal Resting Stations		Light Traps		Night Biting Collections	
	No. Coll.	No. A. quad.	No. Coll.	No. A. quad.	No. Coll.	No. A. quad.
January	585	33	364	6	7	0
February	479	12	366	9	9	0
March	504	2	402	3	10	1
April	1,043	13	265	5	101	0
May	614	212	560	20	170	9
June	3,042	1,372	1,247	450	424	31
July	3,248	15,146	1,390	865	457	119
August	3,572	6,255	1,043	575	473	91
September	4,570	3,428	1,907	637	753	131
Seasonal Total	17,657	26,473	7,544	2,570	2,404	382
Average per Collection		1.50		.34		.16

Form M-4 (Revised)
4th S.C. Lab.

FIG. 1. ADULT MOSQUITO RESTING STATION COLLECTIONS
Weekly Report

Post Avon Park Bombing Range
Avon Park, Florida

Collector Pvt. M. Nateon

Identified by Sgt. R. Arnett

Weekly Period August 8-14, 1943

Date of Identification August 14, 1943

Sta. No.	Area	Date	Kind of Station	ANOPHELES - quad. M	ANOPHELES - quad. F	ANOPHELES - cruc. M	ANOPHELES - cruc. F	CULICINES - C.quin. M	CULICINES - C.quin. F	CULICINES - C.nig. M	CULICINES - C.nig. F	CULICINES - C.con. M	CULICINES - C.con. F	CULICINES - C.fer. M	CULICINES - C.fer. F	CULICINES - A.inf. M	CULICINES - A.inf. F	CULICINES - A.atl. M	CULICINES - A.atl. F	CULICINES - A.tor. M	CULICINES - A.tor. F	CULICINES - A.tas. M	CULICINES - A.tas. F	CULICINES - W.van. M	CULICINES - W.van. F	TOTAL M	TOTAL F
R-1	5	8-12-43	Barrel									1										4					5
R-2	1	8-12-43	Barrel													6						2					8
R-3	5	8-11-43	Barrel											2								1					3
R-4	1	8-11-43	Barrel									2		4		6						2					14
R-5	2	8-12-43	Barrel											1		3						5					9
R-6	4	8-11-43	Barrel									1				1						6					8
R-7	6	8-12-43	Barrel													1						1					2
R-8	7	8-11-43	Tent		2		26	4*	2																	4	30
R-11	7	8-13-43	Bridge									4										1					5
R-12	1	8-11-43	Bridge													3						2					5
R-13	4	8-11-43	Old Bldg.											1		2											3
R-14	7	8-11-43	Tent		5		2		1																		8
R-16	1	8-12-43	Barrel											1								3		1			5
R-17	1	8-12-43	Latrine				2				1					2											5
R-18	8	8-12-43	Building		3				1																		4
R-19	3	8-12-43	Barrel											7		5		1				4					17
R-20	6	8-12-43	Barrel											1		2						2					5
R-21	9	8-13-43	Barrel									5															5
				*Identified by genitalia																							
			TOTALS		10		30	4	4		1		13		17		31		1			33		1		4	141

Form M-5 (Revised)
4th S.C. Lab.

FIG. 2. MOSQUITO LIGHT TRAP COLLECTIONS
Weekly Report

Post Dale Mabry Field
Tallahassee, Florida

Collector Frederick Heisel, Capt., SnC

Identified by Hoogstraal

Weekly Period June 6-12, 1943

Date Identified June 12, 1943

Species	Trap No. 1 Mon. 6/7 M	Trap No. 1 Mon. 6/7 F	Trap No. 1 Wed. 6/9 M	Trap No. 1 Wed. 6/9 F	Trap No. 1 Fri. 6/11 M	Trap No. 1 Fri. 6/11 F	Trap No. 2 Mon. 6/7 M	Trap No. 2 Mon. 6/7 F	Trap No. 2 Wed. 6/9 M	Trap No. 2 Wed. 6/9 F	Trap No. 2 Fri. 6/11 M	Trap No. 2 Fri. 6/11 F	Trap No. 3 Mon. 6/7 M	Trap No. 3 Mon. 6/7 F	Trap No. 3 Wed. 6/9 M	Trap No. 3 Wed. 6/9 F	Trap No. 3 Fri. 6/11 M	Trap No. 3 Fri. 6/11 F	Trap No. ___ Mon. M	Trap No. ___ Mon. F	Trap No. ___ Wed. M	Trap No. ___ Wed. F	Trap No. ___ Fri. M	Trap No. ___ Fri. F	TOTAL FOR WEEK M	TOTAL FOR WEEK F
A. quadrimaculatus				3		2		1				7		3		6		2							0	24
A. crucians				4		7		5				20		3		3		3							0	45
A.																										
A.																										
C. quinquefaeciatus			2*		1*																				3	0
C. sp. damaged				3		2				2		2				2		3							0	14
C. salinarius			2*				1*	1	2*	3					1*	1									6	5
C. erraticus	1*		1*																						2	0
C.(Mel.) sp.				13		2		10		33		23		3		6									0	90
Aedes vexans	1*		1*	2		3		8		11		3				1									2	28
A. sp. damaged										1								2							0	3
A.																										
A.																										
Psor. columbiae							1*	2		1															1	3
P.																										
M. perturbans		3	1	7		1		5		2		12				4									1	34
Theob.																										
Uran. sapphirina										18		3													0	21
																	*Identified by genitalia									
TOTAL	2	3	7	32	1	17	2	32	2	71		70		9	1	23		10							15	267

Form M-1 (Revised)
4th S.C. Lab.

FIG. 3. MOSQUITO BITING RECORDS
Weekly Report

Post Avon Park Bombing Range
Avon Park, Florida

Collector Pfc. William Ellwein

Identified by Cpl. Ross Arnett

Weekly Period May 23-29, 1943

Date of Identification June 1, 1943

Coll. No.	Area	No. Men	Date	Hour of Exposure	Description of Surrounding Area	Species	Total M	Total F
B-36	1	2	25	2045-2115	Open area near pond	A. quadrimaculatus		2
						C. nigripalpus		3
						C. salinarius		3
						C. quinquefasciatus		13
						C. (Mel.) spp.		1
B-37	8	2	26	2115-2130	Open area near hanger	C. quinquefasciatus		14
						C. salinarius		3
B-38	3	2	26	2145-2100	Open area near pond	C. quinquefasciatus		16
						Totals		55

Form M-3 (Revised)
4th S.C. Lab.

FIG. 4. LARVAL MOSQUITO COLLECTIONS
Weekly Report

Post Avon Park Bombing Range
Avon Park, Florida

Collector Cpl. Moes H. Arnett, Jr.

Identified by J. P. Toffaleti

Weekly Period April 11-17, 1943

Date of Identification May 8, 1943

Sta. No.	Area	Date of Coll.	Kind of Breeding Place	Date of Last Larviciding	No. of Dips	NUMBER ANOPHELES Collected: Small	Large	Pupae	TOTAL	Identified: quad.	cruc.		TOTAL	NUMBER CULICINES IDENTIFIED: C. quinque	C. nigrip.	C. salin.	C. restuans	U. sepph.														TOTAL
L-1	9	12	Marsh	None	10	3	3		6		5		5		1																1
L-2	9	12	Marsh	None	10		5		5	3	2		5		1	2																3
L-3	3	12	Creek	None	10	No larvae																										
L-4	5	12	Temp. Pool	(Now Dry)																												
L-5	5	16	Creek	None	10	4	36	1	41		36		36																			
L-6	1	17	Stream	None	4											2	2	2														6
L-7	2	15	Temp. Pool	(Now Dry)																												
L-8	1	16	Pool	None	10									1	2	1																4
L-9	1	14	Ditch	Drip Oiler	10									1		1	1															3
L-10	1	14	Ditch	4-11-43	4										6	2	1															9
L-11	3	13	Borrow Pit	None	10	1	11		12	6	5		11		1	1		5														7
L-12	7	13	Borrow Pit	4-10-43	25	No larvae																										
L-13	1	16	Pool	None	10											9																9
L-14	3	15	Pool	None	10										1	8																9
L-15	1	15	Ditch	(Now Dry)																												
L-16	1	15	Ditch	None	10									2		9	3															14
L-17	2	13	Ditch	None			2		2		2		2		1		5															6
L-18	1	16	Stream	Drip Oiler	10										4	11	5															20
				TOTALS		8	57	1	66	9	50		59	4	19	46	17	5															91

tember of this year, and that the total number of adult and larval specimens identified was 595,886.

In a large program of this nature the problem of maintaining adequate records so that the information is readily accessible and usuable is of primary importance. During the early part of the year mosquito collection forms were revised as shown in Figures 1, 2, 3, and 4.

A large wall chart for tabulating weekly collections of *Anopheles quadrimaculatus* in the different camps is maintained in the laboratory so that weekly comparisons of catches in any camp can be made within a very short time. Weekly summaries, as recorded on the chart, are prepared for the Chief of Medical Services of the Fourth Service Command at the close of each week.

TRAINING OF PERSONNEL

The training of personnel for anti-malaria work has been one of the major phases of the entomological program of the laboratory. During the year, the following short courses have been offered for men engaged in mosquito control work: a two weeks course in mosquito identification, a one week course in mosquito collection and control, and a one week orientation course for officer entomologists being stationed in the Fourth Service Command for the first time.

Mosquito Identification Course. Since January a total of 27 officer entomologists and 25 enlisted men with previous biological or entomological training, representing 36 army installations, have taken the two weeks course in mosquito identification in this laboratory. This course consists largely of instruction and practice in the identification of the mosquitoes of the Southeastern United States and some exotic anopheline species, as well as a series of lectures and conferences on mosquito bionomics and control. Instruction and practice in mosquito dissection has recently been added to this course.

Course in Mosquito Collection and Control. At the request of the Director, Division of Preventive Medicine of the Fourth Service Command, a one week course in mosquito collection and control was organized during April 1943. This course was designed to train enlisted technicians from the different military installations in the Service Command where malaria control work is necessary. The men are given classroom instruction in the recognition of the common anopheline species, the separation of mosquitoes from light trap collections of insects, mosquito control, the preparation of equipment used for collecting mosquitoes, and proper methods of packing, recording, and shipping mosquito specimens to the laboratory for

identification. In this course the men are given actual field experience in setting up mosquito collecting stations and the techniques of collecting mosquitoes, particularly the anopheline species.

A total of 181 enlisted men representing 89 military installations has been given this instruction since April of this year.

Orientation Course for Officer Entomologists. Early during 1943 the Fourth Service Command adopted the policy of allowing all newly assigned entomology officers who have not had previous experience in anti-malaria work to attend a one week orientation course at the Fourth Service Command Laboratory for the purpose of becoming acquainted with the various phases of the mosquito control problem before reporting to their stations. Since the adoption of this policy, 22 officers representing 17 army posts have been given this instruction.

SUMMARY

1. Instructions for making mosquito collections, mapping locations of catches and forms for recording collection data were revised during the early part of 1943. The revised forms are illustrated.

2. Mosquito collections were received from 142 army installations, and a total of 595,886 larval and adult specimens representing 54 species was identified in the Fourth Service Command Medical Laboratory from January through September 1943.

3. A rapid build-up in the abundance of *Anopheles quadrimaculatus* and a peak for the year occurred in July when 10.1 per cent of all adult mosquitoes identified were of his species. The percentage gradually declined during August and September.

4. The average number of *Anopheles quadrimaculatus* per collection in diurnal resting stations was approximately 4½ times as great as the average per light trap collection during the year.

5. An orientation course for officer entomologists, a course in mosquito identification and control for entomologists of both enlisted and officer personnel, and a short course in mosquito collection and control for enlisted men was offered during the year.

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